OXIDOREDUCTASE MOLECULES

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of oxidoreductase molecules and to the use of these sequences in the diagnosis, treatment, and prevention of cell proliferative disorders including cancer, endocrine, metabolic, reproductive, neurological, autoimmune/inflammatory, and viral disorders.

BACKGROUND OF THE INVENTION

Many pathways of biogenesis and biodegradation require oxidoreductase (dehydrogenase or reductase) activity, coupled to the reduction or oxidation of a donor or acceptor cofactor. Potential cofactors include cytochromes, oxygen, disulfide, iron-sulfur proteins, flavin adenine dinucleotide (FAD), and the nicotinamide adenine dinucleotides NAD and NADP (Newsholme, E.A. and Leech, A.R. (1983) Biochemistry for the Medical Sciences, John Wiley and Sons, Chichester, U.K. pp. 779-793).

Reductase activity catalyzes the transfer of electrons between substrate(s) and cofactor(s) with concurrent oxidation of the cofactor. The reverse dehydrogenase reaction catalyzes the reduction of a cofactor and consequent oxidation of the substrate. Oxidoreductase enzymes are a broad superfamily of proteins that catalyze numerous reactions in all cells of organisms ranging from bacteria to plants to humans. These reactions include metabolism of sugar, certain detoxification reactions in the liver, and the synthesis or degradation of fatty acids, amino acids, glucocorticoids, estrogens, androgens, and prostaglandins. Different family members are named according to the direction in which their reactions are typically catalyzed; thus they may be referred to as oxidoreductases, oxidases, reductases, or dehydrogenases. In addition, family members often have distinct cellular localizations, including the cytosol, the plasma membrane, mitochondrial inner or outer membrane, and peroxisomes.

Tetrahydrofolate is a derivatized glutamate molecule that acts as a carrier, providing activated one-carbon units to a wide variety of biosynthetic reactions, including synthesis of purines, pyrimidines, and the amino acid methionine. Tetrahydrofolate is generated by the activity of a holoenzyme complex called tetrahydrofolate synthase, which includes three enzyme activities: tetrahydrofolate dehydrogenase, tetrahydrofolate cyclohydrolase, and tetrahydrofolate synthetase. Thus, tetrahydrofolate dehydrogenase plays an important role in generating building blocks for nucleic and amino acids, crucial to proliferating cells.

Intracellular redox status plays a critical role in the assembly of proteins. A major rate

limiting step in protein folding is the thiol:disulfide exchange necessary for correct protein assembly. Although incubation of reduced, unfolded proteins in buffers containing defined ratios of oxidized and reduced thiols can lead to folding into native conformation, the rate of folding is slow, and the attainment of the native conformation decreases proportionately with protein size and the number of cysteine residues. Certain cellular compartments such as the endoplasmic reticulum of eukaryotes and the periplasmic space of prokaryotes are maintained in a more oxidized state than the surrounding cytosol. Correct disulfide formation can occur in these compartments, but it occurs at a rate that is insufficient for normal cell processes and inadequate for synthesizing secreted proteins.

Protein disulfide isomerases (PDIs), thioredoxins, and glutaredoxins are able to catalyze the formation of disulfide bonds and regulate the redox environment in cells to enable the necessary thiol:disulfide exchanges. Each of these classes of molecules has a somewhat different function, but all belong to a group of disulfide-containing redox proteins that contain a conserved active-site sequence and are ubiquitously distributed in eukaryotes and prokaryotes. PDIs are found in the endoplasmic reticulum of eukaryotes and in the periplasmic space of prokaryotes. PDIs function by exchanging their own disulfide for thiols in a folding peptide chain. In contrast, reduced thioredoxins and glutaredoxins are generally found in the cytoplasm and function by directly reducing disulfides in the substrate proteins. Thioredoxin (Trx), a heat-stable, redox-active protein, contains an active site cysteine disulfide/dithiol. Oxidized thioredoxin, Trx-S, can be reduced to the dithiol form by NADPH and a specific flavoprotein enzyme, thioredoxin reductase. Reduced thioredoxin, Trx-(SH), participates in a number of redox reactions mostly linked to reduction of protein disulfides. Trx and thioredoxin reductase (TR), together with NADPH, form a redox complex in which TR catalyzes the electron transport from NADPH to Trx. The reduced thioredoxin then functions as an electron donor in a wide variety of different metabolic processes.

Disulfide-containing redox proteins not only facilitate disulfide formation, but also regulate and participate in a wide variety of physiological processes. The thioredoxin system serves, for example, as a hydrogen donor for ribonucleotide reductase and controls the activity of enzymes by redox reactions. Mammalian thioredoxin (MT) acts as a hydrogen donor for ribonucleotide reductase and methionine sulfoxide reductase, facilitates refolding of disulfide-containing proteins, and activates the glucocorticoid and interleukin-2 receptors. MT also modulates the DNA binding activity of some transcription factors either directly (TFIIIC, BZLF1, and NF-kB) or indirectly (AP-1) through the nuclear factor Ref-1. The importance of the redox regulation of transcription factors is exemplified by the v-fos oncogene where a point mutation of the thioredoxin-modulated cysteine residue results in constitutive activation of the AP-1 complex. Thioredoxin is secreted by cells using a leaderless pathway and stimulates the proliferation of lymphoid cells, fibroblasts, and a

variety of human solid tumor cell lines. Furthermore, thioredoxin is an essential component of early pregnancy factor, inhibits human immunodeficiency virus expression in macrophages, reduces H_2O_2, scavenges free radicals, and protects cells against oxidative stress (Abate, C. et al., (1990) Science 249: 1157-1161; Rosen, A. et al. (1995) Int. Immunol. 7: 625-633; Tagaya, Y. et al (1989) EMBO J. 8: 757-764; Newman, G. W. (1994) J. Expt. Med. 180: 359-363; and Makino, Y. (1996) J. Clin. Invest. 98: 2469-2477).

Short-chain alcohol dehydrogenases (SCADs) are a family of dehydrogenases that share only 15% to 30% sequence identity, with similarity predominantly in the coenzyme binding domain and the substrate binding domain. In addition to the well-known role in detoxification of ethanol, SCADs are also involved in synthesis and degradation of fatty acids, steroids, and some prostaglandins, and are therefore implicated in a variety of disorders such as lipid storage disease, myopathy, SCAD deficiency, and certain genetic disorders. For example, retinol dehydrogenase is a SCAD-family member (Simon, A. et al. (1995) J. Biol. Chem. 270:1107-1112) that converts retinol to retinal, the precursor of retinoic acid. Retinoic acid, a regulator of differentiation and apoptosis, has been shown to down-regulate genes involved in cell proliferation and inflammation (Chai, X. et al. (1995) J. Biol. Chem. 270:3900-3904). In addition, retinol dehydrogenase has been linked to hereditary eye diseases such as autosomal recessive childhood-onset severe retinal dystrophy (Simon, A. et al. (1996) Genomics 36:424-430).

Propagation of nerve impulses, modulation of cell proliferation and differentiation, induction of the immune response, and tissue homeostasis involve neurotransmitter metabolism (Weiss, B. (1991) Neurotoxicology 12:379-386; Collins, S.M. et al. (1992) Ann. N.Y. Acad. Sci. 664:415-424; Brown, J.K. and Imam, H. (1991) J. Inherit. Metab. Dis. 14:436-458). Many pathways of neurotransmitter metabolism require oxidoreductase activity, coupled to reduction or oxidation of a cofactor, such as NAD^+/NADH (Newsholme, E.A. and Leech, A.R. (1983) Biochemistry for the Medical Sciences, John Wiley and Sons, Chichester, U.K. pp. 779-793). Degradation of catecholamines (epinephrine or norepinephrine) requires alcohol dehydrogenase (in the brain) or aldehyde dehydrogenase (in peripheral tissue). NAD^+-dependent aldehyde dehydrogenase oxidizes 5-hydroxyindole-3-acetate (the product of 5-hydroxytryptamine (serotonin) metabolism) in the brain, blood platelets, liver and pulmonary endothelium (Newsholme, E.A. and Leech, A.R. (supra) p. 786). Other neurotransmitter degradation pathways that utilize NAD^+/NADH-dependent oxidoreductase activity include those of L-DOPA (precursor of dopamine, a neuronal excitatory compound), glycine (an inhibitory neurotransmitter in the brain and spinal cord), histamine (liberated from mast cells during the inflammatory response), and taurine (an inhibitory neurotransmitter of the brain stem, spinal cord and retina) (Newsholme, E.A. and Leech,

A.R. supra, pp. 790, 792). Epigenetic or genetic defects in neurotransmitter metabolic pathways can result in a spectrum of disease states in different tissues including Parkinson's disease and inherited myoclonus (McCance, K.L. and Huether, S.E. (1994) Pathophysiology, Mosby-Year Book, Inc., St. Louis, MO pp. 402-404; Gundlach, A.L. (1990) FASEB J. 4:2761-2766).

3-Hydroxyacyl-CoA dehydrogenase (3HACD) is involved in fatty acid metabolism. It catalyzes the reduction of 3-hydroxyacyl-CoA to 3-oxoacyl-CoA, with concomitant oxidation of NAD to NADH, in the mitochondria and peroxisomes of eukaryotic cells. In peroxisomes, 3HACD and enoyl-CoA hydratase form an enzyme complex called bifunctional enzyme, defects in which are associated with peroxisomal bifunctional enzyme deficiency. This interruption in fatty acid metabolism produces accumulation of very-long chain fatty acids, disrupting development of the brain, bone, and adrenal glands. Infants born with this deficiency typically die within 6 months (Watkins, P. et al. (1989) J. Clin. Invest. 83:771-777; Online Mendelian Inheritance in Man (OMIM), #261515). The neurodegeneration that is characteristic of Alzheimer's disease involves development of extracellular plaques in certain brain regions. A major protein component of these plaques is the peptide amyloid-β (Aβ), which is one of several cleavage products of amyloid precursor protein (APP). 3HACD has been shown to bind the Aβ peptide, and is overexpressed in neurons affected in Alzheimer's disease. In addition, an antibody against 3HACD can block the toxic effects of Aβ in a cell culture model of Alzheimer's disease (Yan, S. et al. (1997) Nature 389:689-695; OMIM, #602057).

17β-hydroxysteroid dehydrogenase (17βHSD6) plays an important role in the regulation of the male reproductive hormone, dihydrotestosterone (DHTT). 17βHSD6 acts to reduce levels of DHTT by oxidizing a precursor of DHTT, 3α-diol, to androsterone which is readily glucuronidated and removed from tissues. 17βHSD6 is active with both androgen and estrogen substrates when expressed in embryonic kidney 293 cells. At least five other isozymes of 17βHSD have been identified that catalyze oxidation and/or reduction reactions in various tissues with preferences for different steroid substrates (Biswas, M.G. and Russell, D.W. (1997) J. Biol. Chem. 272:15959-15966). For example, 17βHSD1 preferentially reduces estradiol and is abundant in the ovary and placenta. 17βHSD2 catalyzes oxidation of androgens and is present in the endometrium and placenta. 17βHSD3 is exclusively a reductive enzyme in the testis (Geissler, W.M. et al. (1994) Nature Genet. 7:34-39). An excess of androgens such as DHTT can contribute to certain disease states such as benign prostatic hyperplasia and prostate cancer.

Steroids, such as estrogen, testosterone, corticosterone, and others, are generated from a common precursor, cholesterol, and are interconverted into one another. A wide variety of enzymes act upon cholesterol, including a number of dehydrogenases. One such dehydrogenase is 3-oxo-5-

α-steroid dehydrogenase (OASD), a microsomal membrane protein highly expressed in prostate and other androgen-responsive tissues. OASD catalyzes the conversion of testosterone into dihydrotestosterone, which is the most potent androgen. Dihydrotestosterone is essential for the formation of the male phenotype during embryogenesis, as well as for proper androgen-mediated growth of tissues such as the prostate and male genitalia. A defect in OASD that prevents the conversion of testosterone into dihydrotestosterone leads to a rare form of male pseudohermaphroditis, characterized by defective formation of the external genitalia (Andersson, S., et al. (1991) Nature 354:159-161; Labrie, F., et al. (1992) Endocrinology 131:1571-1573; OMIM #264600). Thus, OASD plays a central role in sexual differentiation and androgen physiology.

The discovery of new oxidoreductase molecules and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative disorders including cancer, endocrine, metabolic, reproductive, neurological, autoimmune/inflammatory, and viral disorders.

SUMMARY OF THE INVENTION

The invention features substantially purified polypeptides, oxidoreductase molecules, referred to collectively as "OXRE" and individually as "OXRE-1," "OXRE-2," "OXRE-3," "OXRE-4," "OXRE-5," "OXRE-6," "OXRE-7," "OXRE-8," "OXRE-9," "OXRE-10," "OXRE-11,""OXRE-12,""OXRE-13,""OXRE-14," and "OXRE-15." In one aspect, the invention provides a substantially purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof.

The invention further provides a substantially purified variant having at least 90% amino acid identity to at least one of the amino acid sequences selected from the group consisting of SEQ ID NO:1-15 and fragments thereof. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is

complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30 and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least 70% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30 and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30 and fragments thereof.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-15 and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a disorder associated with decreased expression or activity of OXRE, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group consisting of SEQ ID

NO:1-15 and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of OXRE, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-15 and fragments thereof.

BRIEF DESCRIPTION OF THE TABLES

Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding OXRE.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods and algorithms used for identification of OXRE.

Table 3 shows the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; a useful fragment of each nucleic acid; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones encoding OXRE were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze OXRE, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described

herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"OXRE" refers to the amino acid sequences of substantially purified OXRE obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which, when bound to OXRE, increases or prolongs the duration of the effect of OXRE. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of OXRE.

An "allelic variant" is an alternative form of the gene encoding OXRE. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding OXRE include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as OXRE or a polypeptide with at least one functional characteristic of OXRE. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding OXRE, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding OXRE. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent OXRE. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of OXRE is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups

having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of OXRE which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain some biological activity or immunological activity of OXRE. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which, when bound to OXRE, decreases the amount or the duration of the effect of the biological or immunological activity of OXRE. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of OXRE.

The term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, $F(ab')_2$, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind OXRE polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may

be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic OXRE, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" and "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding OXRE or fragments of OXRE may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using the XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to produce the consensus sequence.

The term "correlates with expression of a polynucleotide" indicates that the detection of the

presence of nucleic acids, the same or related to a nucleic acid sequence encoding OXRE, by northern analysis is indicative of the presence of nucleic acids encoding OXRE in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding OXRE.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" and "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR, Madison WI) which creates alignments between two or more sequences according to methods selected by the user, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) Parameters for each method may be the default parameters provided by MEGALIGN or may be specified by the user. The clustal algorithm groups sequences into clusters by examining

the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term "humanized antibody" refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray" refers to an arrangement of distinct polynucleotides on a substrate.

The terms "element" and "array element" in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate" refers to a change in the activity of OXRE. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of OXRE.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which comprise a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:16-30, for example, as distinct from any other sequence in the same genome. For example, a fragment of SEQ ID NO:16-30 is useful in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:16-30 from related polynucleotide sequences. A fragment of SEQ ID NO:16-30 is at least about 15-20 nucleotides in length. The precise length of the fragment of SEQ ID NO:16-30 and the region of SEQ ID NO:16-30 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment. In some cases, a fragment, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" and "operably linked" refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide" refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. "Oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic acids encoding OXRE, or fragments thereof, or OXRE itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of

replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of OXRE polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to OXRE. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

THE INVENTION

The invention is based on the discovery of new human oxidoreductase molecules (OXRE), the polynucleotides encoding OXRE, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative disorders including cancer, endocrine, metabolic, reproductive, neurological, autoimmune/inflammatory, and viral disorders.

Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding OXRE. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each OXRE were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. The clones in column 5 were used to assemble the consensus

nucleotide sequence of each OXRE and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows the identity of each polypeptide; and column 7 shows analytical methods used to identify each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding OXRE. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists a fragment of each nucleotide that is useful as described below. Column 3 lists tissue categories which express OXRE as a fraction of total tissues expressing OXRE. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing OXRE as a fraction of total tissues expressing OXRE. Column 5 lists the vectors used to subclone each cDNA library.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding OXRE were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

Fragments of the nucleotide sequences encoding OXRE, listed in Table 3, Column 2, are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:16-30 and to distinguish between SEQ ID NO:16-30 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides.

The invention also encompasses OXRE variants. A preferred OXRE variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the OXRE amino acid sequence, and which contains at least one functional or structural characteristic of OXRE.

The invention also encompasses polynucleotides which encode OXRE. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:16-30, which encodes OXRE.

The invention also encompasses a variant of a polynucleotide sequence encoding OXRE. In particular, such a variant polynucleotide sequence will have at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding OXRE. A particular aspect of the invention encompasses a

variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:16-30 which has at least about 70%, more preferably at least about 85%, and most preferably at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:16-30. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of OXRE.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding OXRE, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring OXRE, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode OXRE and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring OXRE under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding OXRE or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding OXRE and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode OXRE and OXRE derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding OXRE or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:16-30 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM

NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence

preparation is automated with machines such as the Hamilton MICROLAB 2200 (Hamilton, Reno NV), Peltier thermal cycler 200 (PTC200; MJ Research, Watertown MA) and the ABI CATALYST 800 (Perkin-Elmer). Sequencing is then carried out using either ABI 373 or 377 DNA sequencing systems (Perkin-Elmer), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding OXRE may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode OXRE may be cloned in recombinant DNA molecules that direct expression of OXRE, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express OXRE.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter OXRE-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding OXRE may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 7:215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 7:225-232.) Alternatively, OXRE itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of OXRE, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g, Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by

sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active OXRE, the nucleotide sequences encoding OXRE or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding OXRE. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding OXRE. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding OXRE and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding OXRE and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding OXRE. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus,TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding OXRE. For example, routine cloning,

subcloning, and propagation of polynucleotide sequences encoding OXRE can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or pSPORT1 plasmid (Life Technologies). Ligation of sequences encoding OXRE into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of OXRE are needed, e.g. for the production of antibodies, vectors which direct high level expression of OXRE may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of OXRE. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Grant et al. (1987) Methods Enzymol. 153:516-54; and Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of OXRE. Transcription of sequences encoding OXRE may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding OXRE may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses OXRE in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of OXRE in cell lines is preferred. For example, sequences encoding OXRE can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*$^-$ or *apr*$^-$ cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), ß glucuronidase and its substrate ß-glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding OXRE is inserted within a marker gene sequence, transformed cells containing sequences encoding OXRE can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding OXRE under the

control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding OXRE and that express OXRE may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of OXRE using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on OXRE is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul MN, Sect. IV; Coligan, J. E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding OXRE include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding OXRE, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding OXRE may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the

sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode OXRE may be designed to contain signal sequences which direct secretion of OXRE through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Manassas, VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding OXRE may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric OXRE protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of OXRE activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the OXRE encoding sequence and the heterologous protein sequence, so that OXRE may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled OXRE may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid

precursor, preferably ^{35}S-methionine.

Fragments of OXRE may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra, pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A peptide synthesizer (Perkin-Elmer). Various fragments of OXRE may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of OXRE and oxidoreductase molecules. In addition, the expression of OXRE is closely associated with cell proliferation, cancer, inflammation and immune response, nervous system tissues, and reproductive tissues. Of particular note is the exclusive expression of SEQ ID NO:30 in proliferating brain tissue. Therefore, OXRE appears to play a role in cell proliferative disorders including cancer, endocrine, metabolic, reproductive, neurological, autoimmune/inflammatory, and viral disorders. In the treatment of cell proliferative disorders including cancer, endocrine, metabolic, reproductive, neurological, autoimmune/inflammatory, and viral disorders associated with increased OXRE expression or activity, it is desirable to decrease the expression or activity of OXRE. In the treatment of the above conditions associated with decreased OXRE expression or activity, it is desirable to increase the expression or activity of OXRE.

Therefore, in one embodiment, OXRE or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of OXRE. Examples of such disorders include, but are not limited to, a cell proliferative disorder, such as such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease, myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia; a cancer, such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an endocrine disorder such as disorders of the hypothalamus and pituitary resulting from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular malformations, thrombosis, infections, immunological disorders, and complications due to head trauma; disorders associated with hypopituitarism including hypogonadism, Sheehan syndrome, diabetes insipidus, Kallman's disease, Hand-Schuller-Christian disease, Letterer-Siwe disease,

sarcoidosis, empty sella syndrome, and dwarfism; disorders associated with hyperpituitarism including acromegaly, giantism, and syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) often caused by benign adenoma; disorders associated with hypothyroidism including goiter, myxedema, acute thyroiditis associated with bacterial infection, subacute thyroiditis associated with viral infection, autoimmune thyroiditis (Hashimoto's disease), and cretinism; disorders associated with hyperthyroidism including thyrotoxicosis and its various forms, Grave's disease, pretibial myxedema, toxic multinodular goiter, thyroid carcinoma, and Plummer's disease; disorders associated with hyperparathyroidism including Conn disease (chronic hypercalemia); pancreatic disorders such as Type I or Type II diabetes mellitus and associated complications; disorders associated with the adrenals such as hyperplasia, carcinoma, or adenoma of the adrenal cortex, hypertension associated with alkalosis, amyloidosis, hypokalemia, Cushing's disease, Liddle's syndrome, and Arnold-Healy-Gordon syndrome, pheochromocytoma tumors, and Addison's disease; disorders associated with gonadal steroid hormones such as: in women, abnormal prolactin production, infertility, endometriosis, perturbations of the menstrual cycle, polycystic ovarian disease, hyperprolactinemia, isolated gonadotropin deficiency, amenorrhea, galactorrhea, hermaphroditism, hirsutism and virilization, breast cancer, and, in post-menopausal women, osteoporosis; and, in men, Leydig cell deficiency, male climacteric phase, and germinal cell aplasia, hypergonadal disorders associated with Leydig cell tumors, androgen resistance associated with absence of androgen receptors, syndrome of 5 α-reductase, and gynecomastia; a metabolic disorder, such as Addison's disease, cystic fibrosis, diabetes, fatty hepatocirrhosis, galactosemia, goiter, hyperadrenalism, hypoadrenalism, hyperparathyroidism, hypoparathyroidism, hypercholesterolemia, hyperthyroidism, hypothyroidism hyperlipidemia, hyperlipemia, lipid myopathies, obesity, lipodystrophies, and phenylketonuria, congenital adrenal hyperplasia, pseudovitamin D-deficiency rickets, cerebrotendinous xanthomatosis, and coumarin resistance; a reproductive disorder such as disorders of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders,

progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a viral disorder, such as viral infections, e.g., those caused by adenoviruses (acute respiratory disease, pneumonia), arenaviruses (lymphocytic choriomeningitis), bunyaviruses (Hantavirus), coronaviruses (pneumonia, chronic bronchitis), hepadnaviruses (hepatitis), herpesviruses (herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus), flaviviruses (yellow fever), orthomyxoviruses (influenza), papillomaviruses (cancer), paramyxoviruses (measles, mumps), picornoviruses (rhinovirus, poliovirus, coxsackie-virus), polyomaviruses (BK virus, JC virus), poxviruses (smallpox), reovirus (Colorado tick fever), retroviruses (human immunodeficiency virus,

human T lymphotropic virus), rhabdoviruses (rabies), rotaviruses (gastroenteritis), and togaviruses (encephalitis, rubella).

In another embodiment, a vector capable of expressing OXRE or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of OXRE including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified OXRE in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of OXRE including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of OXRE may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of OXRE including, but not limited to, those listed above.

In a further embodiment, an antagonist of OXRE may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of OXRE. Examples of such disorders include, but are not limited to, those described above. In one aspect, an antibody which specifically binds OXRE may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express OXRE.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding OXRE may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of OXRE including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of OXRE may be produced using methods which are generally known in the art. In particular, purified OXRE may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind OXRE. Antibodies to OXRE may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with OXRE or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to OXRE have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of OXRE amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to OXRE may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce OXRE-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for OXRE may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between OXRE and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering OXRE epitopes is preferred, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for OXRE. Affinity is expressed as an association constant, K_a, which is defined as the molar concentration of OXRE-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple OXRE epitopes, represents the average affinity, or avidity, of the antibodies for OXRE. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular OXRE epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the OXRE-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of OXRE, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington, DC; Liddell, J. E. and Cryer, A. (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is preferred for use in procedures requiring precipitation of OXRE-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g.,

Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding OXRE, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding OXRE may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding OXRE. Thus, complementary molecules or fragments may be used to modulate OXRE activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding OXRE.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding OXRE. (See, e.g., Sambrook, supra; Ausubel, 1995, supra.)

Genes encoding OXRE can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding OXRE. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding OXRE. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage

of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding OXRE.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding OXRE. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of

such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of OXRE, antibodies to OXRE, and mimetics, agonists, antagonists, or inhibitors of OXRE. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar

solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of OXRE, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example OXRE or fragments thereof, antibodies of OXRE, and agonists, antagonists or inhibitors of OXRE, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind OXRE may be used for the diagnosis of disorders characterized by expression of OXRE, or in assays to monitor patients being treated with OXRE or agonists, antagonists, or inhibitors of OXRE. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for OXRE include methods which utilize the antibody and a label to detect OXRE in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring OXRE, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of OXRE expression. Normal or standard values for OXRE expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to OXRE under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of OXRE expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding OXRE may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of OXRE may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of OXRE, and to monitor regulation of OXRE levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding OXRE or closely related molecules may be used to identify nucleic acid sequences which encode OXRE. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding OXRE, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the OXRE encoding sequences. The hybridization probes

of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:16-30 or from genomic sequences including promoters, enhancers, and introns of the OXRE gene.

Means for producing specific hybridization probes for DNAs encoding OXRE include the cloning of polynucleotide sequences encoding OXRE or OXRE derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding OXRE may be used for the diagnosis of disorders associated with expression of OXRE. Examples of such disorders include, but are not limited to, a cell proliferative disorder, such as such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease, myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia; a cancer, such as adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an endocrine disorder such as disorders of the hypothalamus and pituitary resulting from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular malformations, thrombosis, infections, immunological disorders, and complications due to head trauma; disorders associated with hypopituitarism including hypogonadism, Sheehan syndrome, diabetes insipidus, Kallman's disease, Hand-Schuller-Christian disease, Letterer-Siwe disease, sarcoidosis, empty sella syndrome, and dwarfism; disorders associated with hyperpituitarism including acromegaly, giantism, and syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) often caused by benign adenoma; disorders associated with hypothyroidism including goiter, myxedema, acute thyroiditis associated with bacterial infection, subacute thyroiditis associated with viral infection, autoimmune thyroiditis (Hashimoto's disease), and cretinism; disorders associated with hyperthyroidism including thyrotoxicosis and its various forms, Grave's disease, pretibial myxedema, toxic multinodular goiter, thyroid carcinoma, and Plummer's disease; disorders associated with hyperparathyroidism including Conn disease (chronic hypercalemia); pancreatic disorders such as Type I or Type II diabetes mellitus and associated complications; disorders associated with the adrenals such as hyperplasia,

carcinoma, or adenoma of the adrenal cortex, hypertension associated with alkalosis, amyloidosis, hypokalemia, Cushing's disease, Liddle's syndrome, and Arnold-Healy-Gordon syndrome, pheochromocytoma tumors, and Addison's disease; disorders associated with gonadal steroid hormones such as: in women, abnormal prolactin production, infertility, endometriosis, perturbations of the menstrual cycle, polycystic ovarian disease, hyperprolactinemia, isolated gonadotropin deficiency, amenorrhea, galactorrhea, hermaphroditism, hirsutism and virilization, breast cancer, and, in post-menopausal women, osteoporosis; and, in men, Leydig cell deficiency, male climacteric phase, and germinal cell aplasia, hypergonadal disorders associated with Leydig cell tumors, androgen resistance associated with absence of androgen receptors, syndrome of 5 α-reductase, and gynecomastia; a metabolic disorder, such as Addison's disease, cystic fibrosis, diabetes, fatty hepatocirrhosis, galactosemia, goiter, hyperadrenalism, hypoadrenalism, hyperparathyroidism, hypoparathyroidism, hypercholesterolemia, hyperthyroidism, hypothyroidism hyperlipidemia, hyperlipemia, lipid myopathies, obesity, lipodystrophies, and phenylketonuria, congenital adrenal hyperplasia, pseudovitamin D-deficiency rickets, cerebrotendinous xanthomatosis, and coumarin resistance; a reproductive disorder such as disorders of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease; prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome; fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system

disorders, dermatomyositis and polymyositis; inherited, metabolic, endocrine, and toxic myopathies; myasthenia gravis, periodic paralysis; mental disorders including mood, anxiety, and schizophrenic disorders; seasonal affective disorder (SAD); akathesia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a viral disorder, such as viral infections, e.g., those caused by adenoviruses (acute respiratory disease, pneumonia), arenaviruses (lymphocytic choriomeningitis), bunyaviruses (Hantavirus), coronaviruses (pneumonia, chronic bronchitis), hepadnaviruses (hepatitis), herpesviruses (herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus), flaviviruses (yellow fever), orthomyxoviruses (influenza), papillomaviruses (cancer), paramyxoviruses (measles, mumps), picornoviruses (rhinovirus, poliovirus, coxsackie-virus), polyomaviruses (BK virus, JC virus), poxviruses (smallpox), reovirus (Colorado tick fever), retroviruses (human immunodeficiency virus, human T lymphotropic virus), rhabdoviruses (rabies), rotaviruses (gastroenteritis), and togaviruses (encephalitis, rubella). The polynucleotide sequences encoding OXRE may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered OXRE expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding OXRE may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding OXRE may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After

a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding OXRE in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of OXRE, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding OXRE, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding OXRE may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding OXRE, or a fragment of a polynucleotide complementary to the polynucleotide encoding OXRE, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantify the expression of OXRE include radiolabeling

or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding OXRE may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding OXRE on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse,

may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, OXRE, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between OXRE and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with OXRE, or fragments thereof, and washed. Bound OXRE is then detected by methods well known in the art. Purified OXRE can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding OXRE specifically compete with a test compound for binding OXRE. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with OXRE.

In additional embodiments, the nucleotide sequences which encode OXRE may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. [Attorney Docket No. PF-0610 P, filed October 6, 1998], U.S. Ser. No. [Attorney Docket No. PF-0616 P, filed December 2, 1998], and U.S. Ser. No. 60/123,911, are hereby expressly incorporated by reference.

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), pSPORT1 plasmid (Life Technologies), or pINCY (Incyte Pharmaceuticals, Palo Alto CA). Recombinant plasmids were transformed into competent E. coli cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5α, DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a Fluoroskan II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Perkin-Elmer) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Perkin-Elmer) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example V.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and

other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programing, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Str. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:16-30. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is

categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding OXRE occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation/trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

V. Extension of OXRE Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:16-30 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+}, $(NH_4)_2SO_4$, and β-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec;

Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent E. coli cells. Transformed cells were selected on antibiotic-containing media, individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethysulphoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Perkin-Elmer).

In like manner, the nucleotide sequences of SEQ ID NO:16-30 are used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:16-30 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xbal, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography and compared.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-

linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the OXRE-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring OXRE. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of OXRE. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the OXRE-encoding transcript.

IX. Expression of OXRE

Expression and purification of OXRE is achieved using bacterial or virus-based expression systems. For expression of OXRE in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express OXRE upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of OXRE in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding OXRE by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, OXRE is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step,

affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from OXRE at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch 10 and 16). Purified OXRE obtained by these methods can be used directly in the following activity assay.

X. Demonstration of OXRE Activity

For purposes of example, an assay demonstrating the activity of a short-chain alcohol dehydrogenase is described. Essentially the same method is used for other types of oxidoreductases, with suitable substitution of the substrate and electron acceptor. OXRE activity is demonstrated by the oxidation of NADPH to NADP in the presence of substrate (Kunau and Dommes (1978) Eur. J. Biochem. 91:533-544). Substrates include, but are not limited to, all-trans-retinaldehyde and *cis*-4-dienoyl-CoA. OXRE is preincubated for 10 minutes at 37 °C in 60 µM potassium phosphate (pH 7.4), 125 nM NADPH, and 0.2 µM CoA (coenzyme A). The reaction is initiated by addition of the appropriate substrate (12.5 to 150 µM final concentration). The change in absorbance of the reaction at 340 nm, due to the oxidation of NADPH to NADP, is measured using a spectrophotometer at 23 °C. Units of OXRE activity are expressed as µmoles of NADP formed per minute. A reaction lacking OXRE is used as a negative control.

Alternatively, OXRE activity is assayed by measuring the reduction of insulin. Aliquots of OXRE are preincubated at 37 °C for 20 min with 2 µl of 50 mM Hepes, pH 7.6, 100 µg/ml bovine serum albumin, and 2 mM DTT in a total volume of 70 µl. Then, 40 µl of a reaction mixture composed of 200 µl of Hepes (1 M), pH 7.6, 40 µl of EDTA (0.2 M), 40 µl of NADPH (40 mg/ml), and 500 µl of insulin (10 mg/ml) is added. The reaction is initiated with the addition of 10 µl of thioredoxin reductase from calf thymus (3.0 A412 unit), and incubation is continued for 20 min at 37 °C. The reaction rate is followed by monitoring the oxidation of NADPH at 412 nM. The oxidation of NADPH is proportional to the amount of insulin reduction.

XI. Functional Assays

OXRE function is assessed by expressing the sequences encoding OXRE at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors

of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μg of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2 μg of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of OXRE on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding OXRE and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding OXRE and other genes of interest can be analyzed by northern analysis or microarray techniques.

XII. Production of OXRE Specific Antibodies

OXRE substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the OXRE amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for

selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Perkin-Elmer) using fmoc-chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XIII. Purification of Naturally Occurring OXRE Using Specific Antibodies

Naturally occurring or recombinant OXRE is substantially purified by immunoaffinity chromatography using antibodies specific for OXRE. An immunoaffinity column is constructed by covalently coupling anti-OXRE antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing OXRE are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of OXRE (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/OXRE binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and OXRE is collected.

XIV. Identification of Molecules Which Interact with OXRE

OXRE, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled OXRE, washed, and any wells with labeled OXRE complex are assayed. Data obtained using different concentrations of OXRE are used to calculate values for the number, affinity, and association of OXRE with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	16	000746	U937NOT01	000746H, 000746X777, and 002067R1 (U937NOT01), 1304321T1 (PLACNOT02), 967947R1 (BRSTNOT05), SAPA01383F1, SXAA00973D1
2	17	2472577	THP1NOT03	755151R1 (BRAITUT02), 2472577H1 (THP1NOT03), 2476593F6 (SMCANOT01)
3	18	2160405	ENDCNOT02	1579324T6 (DUODNOT01), 1929782F6 (COLNTUT03), 1984568R6 (LUNGAST01), 1984568T6 (LUNGAST01), 2160405H1 (ENDCNOT02), 3088049F6 (HEAONOT03), SBMA01335F1
4	19	2591695	LUNGNOT22	1293773H1 (PGANNOT03), 1981831H1 (LUNGTUT03), 2591695H1 (LUNGNOT22), 2599633T6 (UTRSNOT10), 3079232H1 (BRAIUNT01), 3388467H1 (LUNGTUT17), 3728027F6 (SMCCNON03)
5	20	474100	MMLR1DT01	474100H1 (MMLR1DT01), 831142H1 and 1543031T1 (PROSTUT04), 1579546F6 (DUODNOT01), 1833969T6 (BRAINON01), 2360323H1 (LUNGFET05), 2571957H1 (HIPOAZT01), 3584211F6 (293TF4T01), SAIA02772F1
6	21	1304767	PLACNOT02	737411X28R1 (TONSNOT01), 1304767H1 (PLACNOT02), 1429123T1 (SINTBST01), 1458173R1 (COLNFET02), 1594353T6 (BRAINOT14)
7	22	1465978	PANCTUT02	872915R1 (LUNGAST01), 1465978F6 and 1465978H1 (PANCTUT02), 1865790F6 (PROSNOT19), 2062093R6 and 2132634T6 (OVARNOT03), 2232155F6 (PROSNOT16), 2521685H1 (BRAITUT21), 3037185H1 (SMCCNOT02)
8	23	1635966	UTRSNOT06	108553F1 (AMLBNOT01), 816384R1 (OVARTUT01), 1635966H1 (UTRSNOT06), 3076973H1 (BONEUNT01), SBBA03935F1, SBBA00704F1, SBBA05518F1
9	24	1638410	UTRSNOT06	986854R1 (LVENNOT03), 1297217T1 (BRSTNOT07), 1442549F1 (THYRNOT03), 1542919R1 (PROSTUT04), 1621602F6 (BRAITUT13), 1638410H1 (UTRSNOT06), 1867420F6 (SKINBIT01), 3721081H1 (PENCNOT10), 3770890H1 (BRSTNOT25)

Table 1(cont.)

Protein SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
10	25	1743409	HIPONON01	190557F1 and 190557R1 (SYNORAB01), 1743409H1 and 1743409R6 (HIPONON01), 3725583H1 (BRSTNOT23)
11	26	1803830	SINTNOT13	1611528T6 (COLNTUT06), 1803830H1, 1803830T6, 1803830X12F1 and 1803830X15F1 (SINTNOT13)
12	27	1867333	SKINBIT01	068790F1 and 068790R1 (HUVESTB01), 1209587R1 (BRSTNOT02), 1498674F6 (SINTBST01), 1519693F6 and 1520517F1 (BLADTUT04), 1867333H1 (SKINBIT01), 1958915H1 (CONNNOT01), 2537783H1 (BONRTUT01), 2638196F6 (BONTNOT01), 3039056H1 (BRSTNOT16), 4051183H1 (SINTNOT18), 4228470H1 (BRAMDIT01)
13	28	2906094	THYMNOT05	1442945F6 (THYRNOT03), 1711329X17C1 and 1711654X21C1 (PROSNOT16), 2309777H1 (NGANNOT01), 2906094H1 (THYMNOT05)
14	29	3294314	TLYJINT01	1528021F1 (UCMCL5T01), 2207228T6 (SINTFET03), 3294314H1 (TLYJINT01), 3333445F6 (BRAIFET01)
15	30	4940951	BRAIFEN03	4940951F6, 4940951H1, and 4940951T6 (BRAIFEN03)

Table 2

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
1	280	S68 T119 S128 S247 T257 S92 S209 S221 T257 T277		Thioredoxin domain: R28-E131 Thioredoxin family active site: P36-V78	Thioredoxin	Motifs PFAM PROFILESCAN
2	166	S26 S56 S136 S150 T158		Q2-C162	C1-tetrahydrofolate dehydrogenase (GI901850)	Motifs BLAST BLOCKS PRINTS PFAM
3	319	S61 S67 S111 T167 S216 S218 S250		V9-D283	Lambda crystallin (GI164905)	Motifs BLAST PFAM BLOCKS
4	318	S140 T191 Y149			3-Oxo-5-alpha-steroid dehydrogenase (GI401056)	Motifs BLAST
5	330	T158 S247 T253 S294 S203 S239	N204	Transmembrane region: F117-F136	dTDP-6-deoxy-L-mannose dehydrogenase GI 1314582	BLAST, HMM, MOTIFS, PRINTS
6	266	T60 T201 T235 T257 T72	N134	Short-chain dehydrogenase: K10-P186 G212-V241	Glucose dehydrogenase GI 216268	BLAST, PFAM, HMM, MOTIFS, BLOCKS, PRINTS
7	302	T79 S131 S135 T222 T72 T106 T154 T190 T297		Short-chain dehydrogenase: E39-T216	Retinal short-chain dehydrogenase GI 3450828	BLAST, PFAM, HMM, MOTIFS, BLOCKS, PRINTS
8	300	S33 S286 T52 S95 T198 T267 S103 T151 S187		Short-chain dehydrogenase: E37-G224 Signal Peptide: M1-S18	Retinal short-chain dehydrogenase GI 3450830	BLAST, PFAM HMM, MOTIFS, BLOCKS, PRINTS

Table 2 (cont.)

Protein SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences	Identification	Analytical Methods
9	613	T87 S116 S118 S213 S268 T512 S519 S530 S532 T534 S27 T87 S149 T190 T229 T263 S376 S461 Y95	N186 N350 N468	Pyridine nucleotide-disulphide oxidoreductase: F134-Q523	Putative ferredoxin reductase MOCF GI 3411185	BLAST, PFAM, HMM, MOTIFS, BLOCKS, PRINTS
10	325	T150 T154 S268 T6 T106 S146 Y167	N243	Short-chain dehydrogenase: A53-N243, S194-R222 Transmembrane region: F18-L37	Similar to the insect-type alcohol dehydrogenase GI 1125838	BLAST, PFAM, HMM, MOTIFS, BLOCKS, PRINTS
11	421	T119 T126 S169 S228 T406 T108 T202 T413 Y247			Gamma-Butyrobetaine hydroxylase GI 3746805	BLAST, MOTIFS
12	610	S484 T46 S151 S241 T397 T414 S444 T449 T466 S492 T564 S335 T380 T488 S551	N271	Pyridine nucleotide-disulphide oxidoreductase: V70-E341 Signal Peptide: M1-C18	Pyridine nucleotide-disulphide oxidoreductase	HMM, PFAM, MOTIFS
13	415	S33 S101 S165 T318 S175	N186	Acyl CoA dehydrogenase: L41-R410	Acyl CoA reductase (fadE9) GI 2911026	BLAST, PFAM, HMM, MOTIFS, BLOCKS
14	274	S61 S108 T109 S259 S7 T44 T216 T265		Delta 1-pyrroline-5-carboxylate reductase: R9-A256	Pyrroline-5-carboxylate reductase GI 189498	BLAST, PFAM, HMM, MOTIFS, BLOCKS
15	283	S83 S28 S90 S129 S137 S252 T46 S239 S244	N114	Aldehyde ferredoxin oxidoreductase: P102-N114 Transmembrane region: F260-I279	Aldehyde ferrodoxin oxidoreductase	HMM, BLOCKS

Table 3

Nucleotide SEQ ID NO:	Useful Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
16	112-147		Cell proliferative (0.68) Inflammation/immune (0.26)	pBLUESCRIPT
17	189-218	Cardiovascular (0.500) Hematopoietic/Immune (0.125) Musculoskeletal (0.125)	Cancer (0.625) Inflammation (0.250)	
18	597-626	Reproductive (0.225) Gastrointestinal (0.197) Hematopoietic/Immune (0.127)	Cancer (0.620) Inflammation (0.254)	
19	57-86	Gastrointestinal (0.217) Cardiovascular (0.174) Reproductive (0.130)	Cancer (0.652) Inflammation (0.217)	
20	1028 - 1072	Nervous (0.202) Reproductive (0.177) Gastrointestinal (0.129)	Cancer (0.395) Inflammation (0.290) Cell proliferative (0.161)	PSPORT1
21	651 - 695	Nervous (0.286) Hematopoietic/Immune (0.143) Reproductive (0.143)	Cancer (0.393) Cell proliferative (0.214) Inflammation (0.214)	pINCY
22	619 - 663	Reproductive (0.296) Gastrointestinal (0.185) Nervous (0.141)	Cancer (0.526) Cell proliferative (0.185) Inflammation (0.185)	pINCY
23	1034 - 1078	Reproductive (0.211) Gastrointestinal (0.203) Hematopoietic/Immune (0.148)	Cancer (0.469) Inflammation (0.289) Cell proliferative (0.180)	pINCY
24	768 - 812	Reproductive (0.306) Cardiovascular (0.139) Nervous (0.139)	Cancer (0.500) Inflammation (0.250) Cell proliferative (0.139)	pINCY
25	999 - 1043	Reproductive (0.365) Cardiovascular (0.115) Gastrointestinal (0.096)	Cancer (0.500) Inflammation (0.231) Cell proliferative (0.173)	PSPORT1
26	642 - 686	Reproductive (0.500) Gastrointestinal (0.125) Developmental (0.083)	Cancer (0.542) Cell proliferative (0.167) Inflammation (0.167)	pINCY

Table 3 (cont.)

Nucleotide SEQ ID NO:	Useful Fragment	Tissue Expression (Fraction of Total)	Disease or Condition (Fraction of Total)	Vector
27	56 - 100	Reproductive (0.301) Gastrointestinal (0.221) Nervous (0.106)	Cancer (0.540) Inflammation (0.248) Cell proliferative (0.097)	pINCY
28	1258 - 1302	Reproductive (0.333) Nervous (0.194) Hematopoietic/Immune (0.139)	Cancer (0.583) Cell proliferative (0.194) Inflammation (0.194)	pINCY
29	57 - 101	Developmental (0.214) Nervous (0.214) Hematopoietic/Immune (0.143)	Cancer (0.500) Cell proliferative (0.429) Inflammation (0.143)	pINCY
30	35 - 79	Nervous (1.000)	Cell proliferative (1.000)	pINCY

Table 4

Polynucleotide SEQ ID NO:	Library	Library Comment
16	U937NOT01	The U-937 cDNA library, U937NOT01, was constructed at Stratagene using RNA isolated from the U937 monocyte-like cell line. This cell line (ATCC CRL1593) was established by C. Sundstrom and K. Nilsson in 1974 from cells obtained from the pleural effusion of a 37-year-old Caucasian male with diffuse histiocytic lymphoma.
17	THP1NOT03	Library was constructed using RNA isolated from untreated THP-1 cells. THP-1 (ATCC TIB 202) is a human promonocyte cell line derived from the peripheral blood of a 1-year-old Caucasian male with acute monocytic leukemia.
18	ENDCNOT02	Library was constructed using RNA isolated from dermal microvascular endothelial cells removed from a 30-year-old Caucasian female.
19	LUNGNOT22	Library was constructed using RNA isolated from lung tissue removed from a 58-year-old Caucasian female. The tissue sample used to construct this library was found to have tumor contaminant upon microscopic examination. Pathology for the associated tumor tissue indicated a caseating granuloma. Family history included congestive heart failure, breast cancer, secondary bone cancer, acute myocardial infarction and atherosclerotic coronary artery disease.
20	MMLR1DT01	Library was constructed using RNA isolated from adherent mononuclear cells, which came from a pool of male and female donors. The cells were cultured for 24 hours following Ficoll Hypaque centrifugation.
21	PLACNOT02	Library was constructed using RNA isolated from the placental tissue of a Hispanic female fetus, who was prematurely delivered at 21 weeks' gestation.

Table 4 (cont.)

Polynucleotide SEQ ID NO:	Library	Library Comment
22	PANCTUT02	Library was constructed using RNA isolated from pancreatic tumor tissue removed from a 45-year-old Caucasian female during radical pancreaticoduodenectomy. Pathology indicated a grade 4 anaplastic carcinoma. Family history included benign hypertension, hyperlipidemia and atherosclerotic coronary artery disease.
23	UTRSNOT06	Library was constructed using RNA isolated from myometrial tissue removed from a 50-year-old Caucasian female during a vaginal hysterectomy. Pathology indicated residual atypical complex endometrial hyperplasia. Pathology for the associated tissue removed during dilation and curettage indicated fragments of atypical complex hyperplasia and a single microscopic focus suspicious for grade 1 adenocarcinoma. Patient history included benign breast neoplasm, hypothyroid disease, polypectomy, and arthralgia. Family history included cerebrovascular disease, atherosclerotic coronary artery disease, hyperlipidemia, and chronic hepatitis.
24	UTRSNOT06	Library was constructed using RNA isolated from myometrial tissue removed from a 50-year-old Caucasian female during a vaginal hysterectomy. Pathology indicated residual atypical complex endometrial hyperplasia. Pathology for the associated tissue removed during dilation and curettage indicated fragments of atypical complex hyperplasia and a single microscopic focus suspicious for grade 1 adenocarcinoma. Patient history included benign breast neoplasm, hypothyroid disease, polypectomy, and arthralgia. Family history included cerebrovascular disease, atherosclerotic coronary artery disease, hyperlipidemia, and chronic hepatitis.
25	HIPONON01	This normalized hippocampus library was constructed from 1.13 million independent clones from a hippocampus library. RNA was isolated from the hippocampus tissue of a 72-year-old Caucasian female who died from an intracranial bleed. Patient history included nose cancer, hypertension, and arthritis. The normalization and hybridization conditions were adapted from Soares et al. (PNAS (1994) 91:9928).

Table 4 (cont.)

Polynucleotide SEQ ID NO:	Library	Library Comment
26	SINTNOT13	Library was constructed using RNA isolated from ileum tissue obtained from a 25-year-old Asian female during a partial colectomy and temporary ileostomy. Pathology indicated moderately active chronic ulcerative colitis, involving colonic mucosa from the distal margin to the ascending colon. Family history included hyperlipidemia, depressive disorder, malignant cervical neoplasm, viral hepatitis A, and depressive disorder.
27	SKINBIT01	Library was constructed using RNA isolated from diseased skin tissue of the left lower leg. Patient history included erythema nodosum of the left lower leg.
28	THYMNOT05	Library was constructed using RNA isolated from thymus tissue removed from a 3-year-old Hispanic male during thymectomy and closure of a patent ductus arteriosus. The patient presented with severe pulmonary stenosis and cyanosis. Patient history included cardiac catheterization, echocardiogram, and corrective cardiac surgeries.
29	TLYJINT01	Library was constructed using RNA isolated from a Jurkat cell line derived from the T cells of a male. The cells were treated for 18 hours with 50 ng/ml phorbol ester and 1 μM calcium ionophore. Patient history included acute T-cell leukemia.
30	BRAIFEN03	This normalized fetal brain tissue library was constructed from 3.26 million independent clones from a fetal brain library. Starting RNA was made from brain tissue removed from a Caucasian male fetus with a hypoplastic left heart stillborn after 23 weeks' gestation. The library was normalized in two rounds (with 48 hour reannealing hybridizations) using conditions adapted from Soares et al. (PNAS (1994) 91:9228) and Bonaldo et al. (Genome Research 6 (1996):791).

TABLE 5

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Perkin-Elmer Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25: 3389-3402.	*ESTs:* Probability value= 1.0E-8 or less *Full Length sequences*: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad Sci. 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183: 63-98; and Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489.	*ESTs:* fasta E value=1.06E-6 *Assembled ESTs*: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less *Full Length sequences:* fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS and PRINTS databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S and J.G. Henikoff, Nucl. Acid Res., 19:6565-72, 1991. J.G. Henikoff and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37: 417-424.	Score=1000 or greater; Ratio of Score/Strength = 0.75 or larger; and Probability value= 1.0E-3 or less
PFAM	A Hidden Markov Models-based application useful for protein family search.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322.	Score=10-50 bits, depending on individual protein families

TABLE 5 cont.

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25: 217-221.	Score= 4.0 or greater
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M. S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M. S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12: 431-439.	Score=5 or greater
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch et al. supra; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	